

CLAIMS

1. A method of preparing particles for immunoassays, comprising:
reacting particles comprising carboxylate groups with N-hydroxysuccinimide or N-hydroxysulfosuccinimide and with a carbodiimide
5 coupling reagent to provide activated particles comprising succinimide ester groups;

contacting said activated particles with antibodies to provide sensitized particles comprising covalently bound antibodies and residual succinimide esters; and

10 treating said sensitized particles in an aqueous mixture with an amine compound of formula (I):



wherein $-\text{X}$ is selected from the group consisting of $-\text{NH}_2$, $-\text{OH}$, and $-\text{CO}_2\text{CH}_2\text{CH}_3$; and

15 R is selected from the group consisting of an alkyl group and an alkyl ether group;

wherein, when $-\text{X}$ is $-\text{NH}_2$ or $-\text{CO}_2\text{CH}_2\text{CH}_3$, R comprises from 1 to 20 carbon atoms; and when $-\text{X}$ is $-\text{OH}$, R comprises from 4 to 20 carbon atoms.

20 2. The method of claim 1, wherein

$-\text{X}$ is selected from the group consisting of $-\text{OH}$ and $-\text{NH}_2$; and

R is an alkyl ether group comprising from 4 to 20 carbon atoms and from 1 to 9 oxygen atoms.

25 3. The method of claim 1, wherein the amine compound is selected from the group consisting of glycine ethyl ester; 2-(aminoethoxy)ethanol; 2,2'-(ethylenedioxy)bisethylamine; and 4,7,10-trioxa-1,3-tridecanediamine.

4. The method of claim 1, wherein the ratio of equivalents of amine compound to equivalents of carboxylate groups is at least 50.

5. The method of claim 1, wherein the ratio of equivalents of amine compound to equivalents of carboxylate groups is at least 100.

6. The method of claim 1, wherein the ratio of equivalents of amine compound to equivalents of carboxylate groups is at least 200.

5 7. The method of claim 1, wherein the aqueous mixture has a pH of at least 7.0.

8. The method of claim 1, wherein the particles covalently bind less than 0.35 milligrams per square meter of non-specific protein when contacted with serum.

10 9. The method of claim 1, wherein the particles covalently bind less than 0.30 milligrams per square meter of non-specific protein when contacted with serum.

15 10. The method of claim 1, wherein the particles covalently bind less than 0.20 milligrams per square meter of non-specific protein when contacted with serum.

11. The method of claim 1, wherein the particles covalently bind less than 0.10 milligrams per square meter of non-specific protein when contacted with serum.

20 12. The method of claim 1, wherein the particles covalently bind less than 0.05 milligrams per square meter of non-specific protein when contacted with serum.

13. The method of claim 1, wherein the particles physically adsorb less than 3 milligrams per square meter of non-specific protein when contacted with serum.

25 14. The method of claim 1, wherein the particles physically adsorb less than 2 milligrams per square meter of non-specific protein when contacted with serum.

15. The method of claim 1, wherein the particles physically adsorb less than 1 milligram per square meter of non-specific protein when contacted with serum.

16. A sensitized particle for use in immunoassays, comprising:

5 a particle comprising a surface;

at least one antibody bound to the surface through a covalent

bond; and

the reaction product of a succinimide ester and an amine

compound of formula (I) on the surface;

$$\text{H}_2\text{N}-\text{R}-\text{X} \quad (\text{I})$$

wherein $-X$ is selected from the group consisting of $-NH_2$,

-OH, and -CO₂CH₂CH₃; and

R is selected from the group consisting of an alkyl group

and an alkyl ether group;

wherein, when $-X$ is $-\text{NH}_2$ or $-\text{CO}_2\text{CH}_2\text{CH}_3$, R comprises

from 1 to 20 carbon atoms; and when $-X$ is $-OH$, R comprises from 4 to 20 carbon atoms.

17. The sensitized particle of claim 16, wherein

-X is selected from the group consisting of -OH and -NH₂; and

R is an alkyl ether group comprising from 4 to 20 carbon atoms

20 R is an alkyl ether group comprising from 4 to 20 carbon atoms and from 1 to 9 oxygen atoms.

18. The sensitized particle of claim 16, wherein the amine

compound is selected from the group consisting of glycine ethyl ester; 2-(aminoethoxy)ethanol; 2,2'-(ethylenedioxy)bisethylamine; and 4,7,10-trio-

1,3-tridecanediamine.

19. The sensitized particle of claim 16, further comprising BSA on the surface.

20. The sensitized particle of claim 16, wherein the particle comprising a surface is selected from the group consisting of gold particles, ceramic particles, and polymer particles.

5 21. The sensitized particle of claim 16, wherein the particles covalently bind less than 0.35 milligrams per square meter of non-specific protein when contacted with serum.

22. The sensitized particle of claim 16, wherein the particles covalently bind less than 0.30 milligrams per square meter of non-specific protein when contacted with serum.

10 23. The sensitized particle of claim 16, wherein the particles covalently bind less than 0.20 milligrams per square meter of non-specific protein when contacted with serum.

15 24. The sensitized particle of claim 16, wherein the particles covalently bind less than 0.10 milligrams per square meter of non-specific protein when contacted with serum.

25. The sensitized particle of claim 16, wherein the particles covalently bind less than 0.05 milligrams per square meter of non-specific protein when contacted with serum.

20 26. The sensitized particle of claim 16, wherein the particles physically adsorb less than 3 milligrams per square meter of non-specific protein when contacted with serum.

27. The sensitized particle of claim 16, wherein the particles physically adsorb less than 2 milligrams per square meter of non-specific protein when contacted with serum.

25 28. The sensitized particle of claim 16, wherein the particles physically adsorb less than 1 milligram per square meter of non-specific protein when contacted with serum.

40052005-140201

29. A particle for use in immunoassays, comprising:
a polymer particle comprising a surface;
at least one antibody bound to the surface through a covalent
bond;
- 5 BSA on the surface; and
the reaction product of a succinimide ester and an amine
compound on the surface;
wherein the amine compound is selected from the group
consisting of glycine ethyl ester; 2-(aminoethoxy)ethanol; 2,2'-
10 (ethylenedioxy)bisethylamine; and 4,7,10-trioxa-1,3-tridecanediamine;
wherein the particles covalently bind less than 0.35
milligrams per square meter of non-specific protein when contacted
with serum; and
wherein the particles physically adsorb less than 2
15 milligrams per square meter of non-specific protein when contacted
with serum.
30. A reagent, comprising:
a plurality of particles;
each of said particles comprising a surface;
an antibody bound to the surface through a covalent bond; and
20 the reaction product of a succinimide ester and an amine
compound of formula (I) on the surface;
$$\text{H}_2\text{N}-\text{R}-\text{X} \quad (\text{I});$$

wherein $-\text{X}$ is selected from the group consisting of $-\text{NH}_2$,
25 $-\text{OH}$, and $-\text{CO}_2\text{CH}_2\text{CH}_3$; and
R is selected from the group consisting of an alkyl group
and an alkyl ether group;
wherein, when $-\text{X}$ is $-\text{NH}_2$ or $-\text{CO}_2\text{CH}_2\text{CH}_3$, R comprises
30 from 1 to 20 carbon atoms; and when $-\text{X}$ is $-\text{OH}$, R comprises from 4
to 20 carbon atoms.

31. The reagent of claim 30, wherein
-X is selected from the group consisting of -OH and -NH₂; and
R is an alkyl ether group comprising from 4 to 20 carbon atoms
and from 1 to 9 oxygen atoms.
- 5 32. The reagent of claim 30, wherein the amine compound is
selected from the group consisting of glycine ethyl ester; 2-
(aminoethoxy)ethanol; 2,2'-(ethylenedioxy)bisethylamine; and 4,7,10-trioxa-
1,3-tridecanediamine.
- 10 33. An assay method for determining an antigen, comprising:
 combining a sample suspected of containing said antigen with
the reagent of claim 30,
 the reagent comprising the antibody of said antigen, and
 the reagent capable of forming a detectable complex with said antigen;
 and
15 determining the presence or amount of said detectable complex
as a measure of said antigen in said sample.
34. A test kit, comprising the reagent of claim 30.

20